Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation.

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Public Summary:

Scientific Abstract:
Current approaches for hematopoietic stem cell (HSC) and organ transplantation are limited by donor and host-mediated immune responses to allo-antigens. Application of these therapies is limited by the toxicity of preparative and post-transplant immunosuppressive regimens and a shortage of appropriate HLA-matched donors. We have been exploring two complementary approaches for genetically modifying donor cells that achieve long-term suppression of cellular proteins that elicit host immune responses to mismatched donor antigens, and provide a selective advantage to genetically engineered donor cells after transplantation. The first approach is based on recent advances that make feasible targeted down-regulation of HLA expression. Suppression of HLA expression could help to overcome limitations imposed by extensive HLA polymorphisms that restrict the availability of suitable donors. Accordingly, we have recently investigated whether knockdown of HLA by RNA interference (RNAi) enables allogeneic cells to evade immune recognition. For efficient and stable delivery of short hairpin-type RNAi constructs (shRNA), we employed lentivirus-based gene transfer vectors that integrate into genomic DNA, thereby permanently modifying transduced donor cells. Lentivirus-mediated delivery of shRNA targeting pan-Class I and allele-specific HLA achieved efficient and dose-dependent reduction in surface expression of HLA in human cells, and enhanced resistance to allo-reactive T lymphocyte-mediated cytotoxicity, while avoiding non-MHC restricted killing. Complementary strategies for genetic engineering of HSC that would provide a selective advantage for transplanted donor cells and enable successful engraftment with less toxic preparative and immunosuppressive regimens would increase the numbers of individuals to whom HLA suppression therapy could be offered. Our second strategy is to provide a mechanism for in vivo selection of genetically modified HSC and other donor cells. We have uniquely combined transplantation during the neonatal period, when tolerance may be more readily achieved, with a positive selection strategy for in vivo amplification of drug-resistant donor HSC. This model system enables the evaluation of mechanisms of tolerance induction to neo-antigens, and allogeneic stem cells during immune ontogeny. HSC are transduced ex vivo by lentivirus-mediated gene transfer of P140K-O(6)-methylguanine-methyltransferase (MGMT(P140K)). The MGMT(P140K) DNA repair enzyme confers resistance to benzylguanine, an inhibitor of endogenous MGMT, and to chloroethylating agents such as BCNU. In vivo chemoselection enables enrichment of donor cells at the stem cell level. Using complementary approaches of in vivo chemoselection and RNAi-induced silencing of HLA expression may enable the generation of histocompatibility-enhanced, and eventually, perhaps "universally" compatible cellular grafts.